34th Annual International Conference of the IEEE EMBS
San Diego, California USA, 28 August - 1 September, 2012

EEG-based Detection of Awakening from Isoflurane Anesthesia in Rats

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***Abstract*—In animal studies, reliable measures for depth of anesthesia are frequently required. Previous findings suggest that the continuous depth of anesthesia indices developed for humans might not be adequate for rats whose EEG changes during anesthesia represent more of quick transitions between discrete states. In this paper, the automatic EEG-based detection of awakening from anesthesia was studied in rats. An algorithm based on Bayesian Information Criterion (BIC) is proposed for the assessment of the switch-like change in the signal characteristics occurring just before the awakening. The method was tested with EEGs recorded from ten rats recovering from isoflurane anesthesia. The algorithm was shown to be able to detect the sudden change in the EEG related to the moment of awakening with a precision comparable to careful visual inspection. Our findings suggest that monitoring such signal changes may offer an interesting alternative to the application of continuous depth of anesthesia indices when avoiding the awakening of the animal during e.g. a clinical experiment.**

I. Introduction

WHEN it comes to estimating depth of anesthesia in humans, electroencephalogram (EEG) has proved its value. Increasing the concentration of anesthetics in the blood produces a continuum of EEG changes. With widely used GABAergic drugs, such as propofol and isoflurane, a characteristic frequency progression pattern is seen. The changes begin with an increase of the high frequency (> 20 Hz) activity, followed by a decrease of the high frequency activity and increase of the middle frequency (10-20 Hz) activity. As the anesthesia deepens, the activity shifts towards the low frequencies (0.5-5 Hz) while the amplitude of the signal increases [1], [2]. Finally, begins the so-called burst suppression pattern in which high activity bursts and isoelectric suppression periods take turns. Compared to the induction, the EEG changes occur in a reversed order during emergence [3], [4]. The above-described signal characteristics have been used in several quantitative parameters proposed for depth of anesthesia measurement.

In animal studies, reliable measures for depth of anesthesia are frequently required. The anesthetic state of the animal is often of interest during a clinical experiment. The measures would also be applicable, for example, in the studies of pharmacological properties of anesthetic drugs in the early phase of drug development. Consecutively, the EEG-based depth of anesthesia estimation has lately been under active research in rats. Generally, the problem has been approached by applying the quantitative depth of anesthesia parameters originally developed for humans into rat experiment [5]–[8].

In our recent publications, the EEG spectral changes and their relation to the clinical signs during anesthesia have been studied in both humans and rats [9]–[11]. In addition, the behavior of different quantitative spectral parameters has been investigated [12], [11]. The fundamental signal characteristics related to deepening anesthesia, that is, the shift from low-amplitude high-frequency signal to high-amplitude low-frequency signal, were found to be comparable between species. However, the above-described smooth frequency progression pattern was not apparent in rats. Instead, during awakening, a switch-like behavior in the signal characteristics was observed just before the first spontaneous movement. This finding suggests that the continuous depth of anesthesia indices developed for humans might not be adequate for rats whose EEG changes during anesthesia represent more of quick transitions between discrete states.

In this paper, the automatic EEG-based detection of awakening from anesthesia is studied in rats. An algorithm based on Bayesian Information Criterion (BIC) is proposed for the assessment of the switch-like change in the signal characteristics occurring just before the awakening. The method is tested with EEGs recorded from ten rats recovering from isoflurane anesthesia. The paper is organized as follows. Section II explains the BIC-based change detection algorithm as well as the experimental protocol and data acquisition procedure. The results are presented in Section III. In Section IV, the conclusions and a short discussion about the results are given.

II. Materials and Methods

A. Experimental Protocol and Data Acquisition

The Animal Care and Use Committee of the Johns Hopkins Medical Institutions approved the experimental

This work was supported in part by grants 09SDG2110140 from the American Heart Association and RO1 HL071568 from the National Institute of Health.

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protocol used in this study. Three epidural screw electrodes (Plastics One, Roanoke, VA) for EEG recording were implanted in the skull of ten adult male Wistar rats (312-348 g). For two of the electrodes, the implantation was performed 1.5 mm anterior to bregma symmetrically over the left and right frontal lobes with 3 mm distance from each other. The last electrode, serving as a reference, was implanted in the sagittal midline 1.5 mm posterior to bregma. The electrodes, lead wires, and the exposed portion of the skull were covered using dental cement. The animals were anaesthetized using isoflurane mixed with 50%/50% oxygen/nitrogen during the implantation procedure. A standard precision anesthesia vaporizer (Penlon Sigma Delta, Penlon LTd., Abingdon, Oxon, UK) providing a gradient concentration of gas anesthetics was used. To guarantee appropriate local anesthesia, subcutaneous injection of lidocaine was used to supplement isoflurane prior to the skin incision.

The experimental protocol was carried out approximately a week after the electrode implantation. The rats were anesthetized using 5% isoflurane mixed with 50%/50% oxygen/nitrogen. The oxygen/nitrogen concentration was kept fixed throughout the experiment. After the induction of anesthesia, the isoflurane concentration was decreased to 2% and continued through a nose cone. Two needle electrodes were attached into the rat's forepaws for ECG recording and the wire for EEG recording was attached to the implanted electrodes. A baseline recording was then performed by keeping the isoflurane concentration in 2%. After ten minutes, the reduction of the concentration was carried out in a step-like manner with a speed of 0.05%/min. The beginning of withdrawal reflex was monitored by pinching the interdigital fold of the rat's right hind limb every 60 s during the isoflurane concentration reduction. The spontaneous movement of the rat was assessed as well. The first spontaneous movement was defined as the moment of awakening, after which the isoflurane concentration was turned back to 2% for 20 min. After this, the recording was stopped, the nose cone was taken off, and the electrode wires were removed.

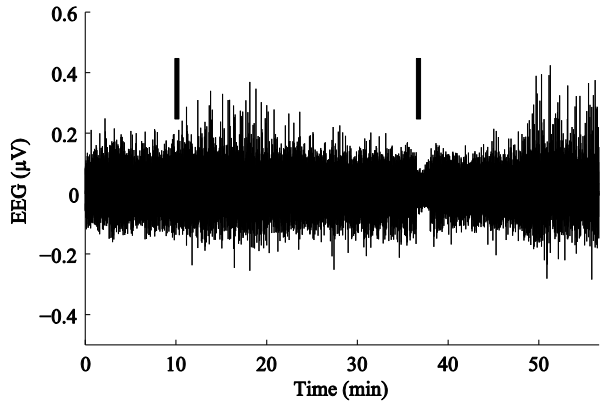

Fig. 1. An example of EEG (Rat 3) during the experimental protocol. The recording begins at Time = 0. The vertical lines indicate the beginning of the isoflurane concentration reduction and the moment of awakening from left to right, respectively.

The EEG was recorded during the experiment with the Tucker Davis Technologies (TDT, Alachua, FL) System 3 data acquisition system with a sampling rate of 305.2 Hz. The signals, recorded using the bipolar montages left frontal-reference and right frontal-reference, were bandpass filtered between 1 Hz and 150 Hz. The 60 Hz AC noise was removed with a second order Butterworth filter. As the signals recorded from the two bipolar montages resembled closely each other, only the EEGs from left frontal-reference were used in the analysis.

All the EEG signal processing was performed with the Matlab technical computing language (The MathWorks Inc., Natick, MA).

B. BIC Algorithm

An algorithm based on BIC [13] is proposed for the detection of the change in the EEG characteristics occurring just before the awakening. The BIC defines a criterion for model selection where few prior assumptions are made. The model priors are assumed as constants and the data distributions are in an exponential family. Due to the asymptotic nature of BIC, a large sample size is required. The EEG signal is assumed as a multivariate Gaussian distribution.

A multiple change detection algorithm, originally developed for speech segmentation [14], was adapted for EEG model change detection. In the algorithm, a growing window of data is searched using BIC to determine whether the data is better explained coming from the same model or by dividing into to sub-windows with different models. For computational efficiency the BIC change detection is performed in coarse steps of a chosen initial window size. The algorithm begins from a window of twice the initial window size, and the window is grown by the initial window size whenever a change is not detected. Furthermore, if a change is not detected in a predefined maximum window size then the oldest initial window size segment is also dropped whenever the window is grown. When a prospective change is detected, to increase the accuracy of the coarse BIC algorithm, a symmetric Kullback-Leibler divergence (KL2) [15] calculation is also implemented. The KL2 is computed in fixed size sliding windows in one sample steps around the prospective change point (initial BIC window size local search area). The maximum peak in the local KL2 curve is then selected as the change point. Then the algorithm is reset to the starting configuration and continued from the change point. The sensitivity of the algorithm can be controlled with a single threshold value.

For application of the algorithm, the EEGs were filtered with seven FIR bandpass filters. The following passbands were used: 1-4 Hz (delta), 5-8 Hz (theta), 9-12 Hz (alpha), 13-25 Hz (gamma1), 26-50 Hz (gamma2), and 51-100 Hz (gamma3). The signals were normalized to have zero mean and unit standard deviation. The algorithm was then applied to the seven-dimensional data using a threshold value of 13.3 (1.9 × the number of coefficients) and an initial widow size of 3 s.

III. Results

Fig. 1 presents an example of EEG recorded during the experimental protocol. A significant decrease in the signal amplitude is clearly seen related to the moment of awakening. The changes in the signal characteristics are illustrated in more detail in Fig. 2 which, in addition to the EEG itself, presents the behavior of the relative powers in different frequency bands of the signal. The sudden decrease of the power in low frequencies, i.e. delta and theta bands, is supplemented with the increase of power in high frequencies, seen especially in the gamma2 and gamma3 bands.

The performance of the BIC algorithm is illustrated in Fig. 3. The switch-like change in the EEG characteristics, occurring approximately six seconds before the moment of awakening is detected by BIC while the exact time point is indicated clearly by a peak in KL2. The algorithm was applied to the EEGs of all ten rats and the results are presented in Table I. For comparison, the signals were carefully manually inspected for changes occurring just before the moment of awakening. The results of the manual and automatic detections are well in line. In majority of the rats whose signal quality was adequate for the analysis, the time difference was only few seconds. For Rat 8, the algorithm detected a change 19 s before the manual detection. Only in one rat (Rat 4) the algorithm seemed to fail. On the other hand, for Rat 1, the algorithm was able to detect change even though the signal quality was too low for the manual detection. Overall, the BIC algorithm seems to be able to detect the sudden change in the EEG related to the moment of awakening with a precision comparable to careful visual inspection.

TABLE I
CHANGE DETECTED IN EEG

	Manual	Automatic	Difference
Rat 1	*	–49	–
Rat 2	–18	–17	1
Rat 3	–6	–5	1
Rat 4	–48	***	–
Rat 5	*	***	–
Rat 6	**	***	–
Rat 7	–24	–29	–5
Rat 8	–18	–37	–19
Rat 9	–42	–43	–1
Rat 10	–6	–5	1

The times when a switch-like change in the EEG characteristics was detected manually and automatically using the BIC algorithm. The values are given in seconds compared to the moment of awakening. The time difference between manual and automatic detection is also given. * Due to the poor quality of the signal, the change was not observable. ** No clear change in the EEG was observed. *** No change was observed before the moment of awakening.

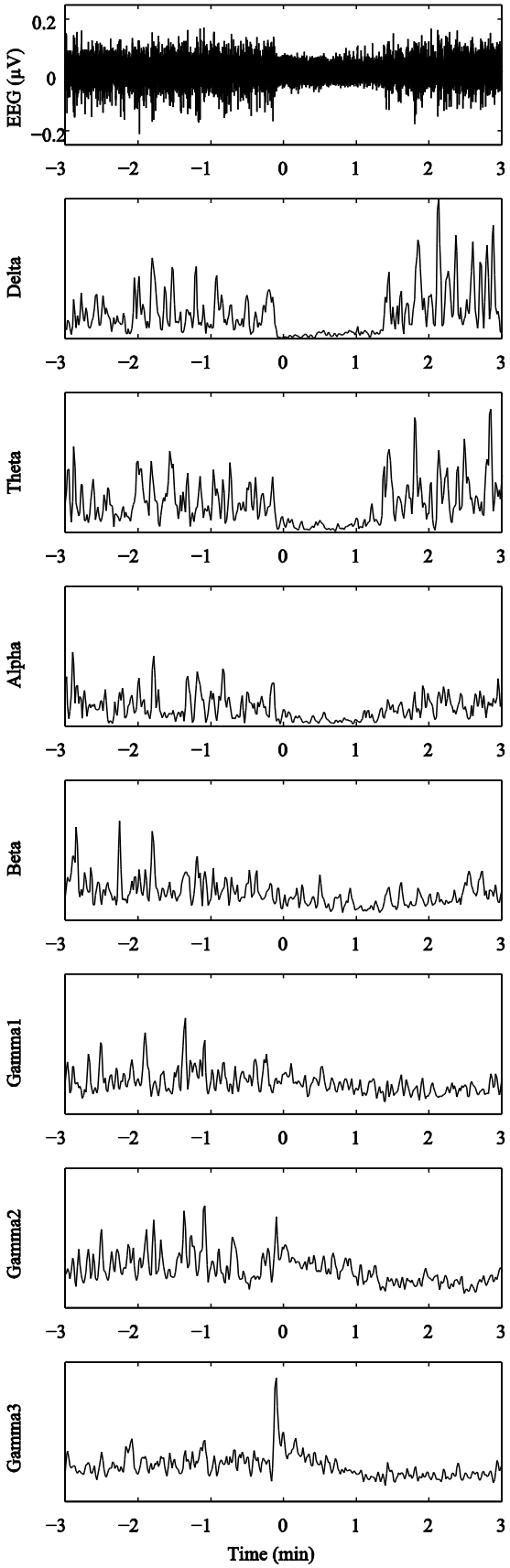

Fig. 2. An example of EEG (Rat 3) during the awakening from anesthesia and the relative powers in different frequency bands of the signal. Time = 0 indicates the moment of awakening. Powers are presented in arbitrary units. See text for details.

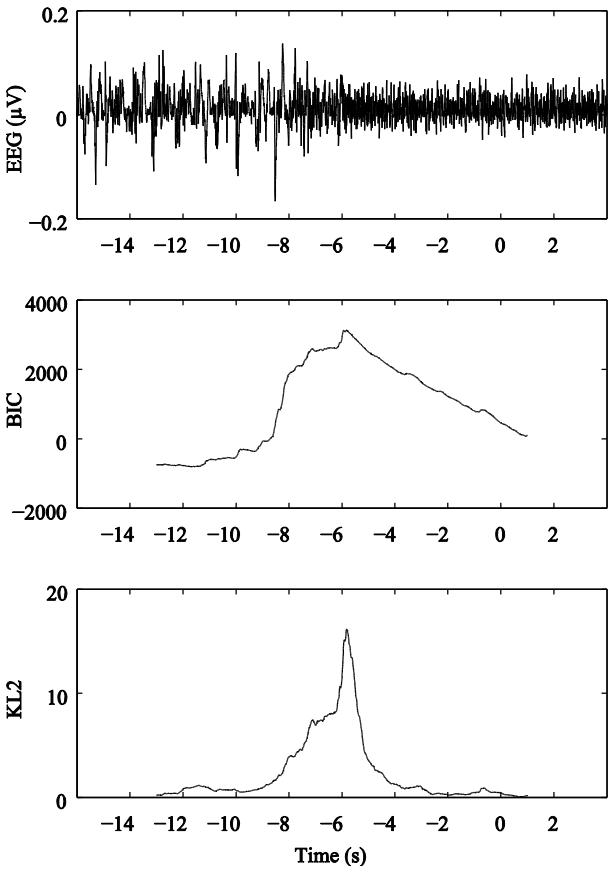

Fig. 3. An example of EEG (Rat 3) during the awakening from anesthesia, the result of algorithm based on Bayesian Information Criterion (BIC), and the symmetric Kullback-Leibler divergence (KL2). BIC is able to locate the switch-like change in the signal characteristic at Time = -6. The change is indicated even more clearly by KL2. To be more illustrative, the BIC curve is calculated using a shorter time window step in the figure than in the actual study.

IV. Conclusions and Discussion

In this paper, the automatic EEG-based detection of awakening from anesthesia is studied in rats. An algorithm based on BIC was proposed for the assessment of the switch-like change in the signal characteristics occurring just before the awakening. The method was tested with EEGs recorded from ten rats recovering from isoflurane anesthesia. The algorithm was able to detect the sudden change in the EEG related to the moment of awakening with a precision comparable to careful visual inspection.

Lately, EEG-based depth of anesthesia estimation in rats has been under active research. The focus has been on determining the anesthetic drug effect by applying the quantitative depth of anesthesia parameters originally developed for humans to rat experiments. For example, approximate entropy, MPF, and SEF95% parameters have been used for this [5], [16], [17]. Like in human studies, the performances of different parameters have generally been compared using the prediction probability statistical analysis [18] in which the monotonicity of certain parameter during continuously increasing/decreasing anesthetic level is quantified. While the interest has been on different computational parameters, little attention seems to be paid to the actual characteristics of EEG. Our findings suggest that monitoring the sudden changes in the signal may offer an interesting alternative to the application of continuous depth of anesthesia indices when avoiding the awakening of the animal during e.g. a clinical experiment.

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